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14. ABSTRACT

The major goal of this project is to determine if the rhodacyanine analog dye, MKT-077, can be used to inhibit breast cancer cell oxygen metabolism and raise tumor oxygen levels, thereby radiosensitizing the tumor. In the first year, we have performed in vitro experiments to determine drug uptake and subsequent MKT-077-induced metabolic inhibition in human MDA-MB 231 cells, as outlined in Tasks 1 and 2. We originally proposed to do this work in our multicellular layer (MCL) model of tumor parenchyma, but we had difficulty growing MCLs from this cell line consistently. Therefore, we have begun determining MKT-077 drug uptake and metabolic inhibition using MDA-MB 231 monolayers and cell suspensions. This approach has been successful, and we have been able to show that the cells rapidly take up the drug, which inhibits cellular oxygen metabolism by 22% at a dose of 4 µg/ml. We are currently completing the drug uptake studies and determining the inhibition caused by other doses. In addition, we have begun the in vivo work in nude rats. We have determined that the rats can tolerate a dose of 10 mg/kg MKT-077 infused at 1.25 mg/kg/min. Soon we will begin measuring MKT-077-induced oxygen changes in orthotopic xenografts in vivo.

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Breast cancer, radiosensitizer, metabolic inhibition, MKT-077, tumor oxygenation

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INTRODUCTION

The driving hypothesis behind this project is that the rhodacyanine dye analog MKT-077 can be used to inhibit breast cancer cell respiration, leading to increased oxygen levels within the tumor and a resultant increase in tumor radiation response. This central hypothesis is built upon the following rationale. First, the response of tumors to radiation therapy is dependent upon oxygen levels,[11] and tumors with low oxygen levels (i.e., hypoxic tumors) are radioresistant.[12] Second, over 60% of human breast tumors are severely hypoxic.[23-25] Third, of the two methods to increase oxygen levels, inhibition of tumor cell oxygen consumption is theoretically a better method than increasing the oxygen supply.[18, 19] Fourth, MKT-077 has been shown to inhibit mitochondrial respiration in some cancer cell types.[16] Therefore, we expect to be able to decrease oxygen consumption and increase the oxygen levels in breast carcinoma by infusing MKT-077. If oxygen can be increased, then infusion of MKT-077 before radiotherapy should increase radiation response and permit lower levels of radiation to be used for preoperative treatment of locally advanced breast cancer (LABC). Lower radiation doses would result in less collateral damage to normal tissue and better cosmetic outcome. In addition, it might be possible to shorten treatment schedules, which would be a benefit to both patients and physicians. Finally, the use of the radiosensitizer could result in better locoregional control in LABC patients. This could increase the number of these women eligible for breast conserving treatment and lead to their improved survival. The use of MKT-077 in combination with radiotherapy should also be useful in patients with early stage breast cancer, who are receiving radiation as part of breast conserving treatment[20, 26] or locoregional post-mastectomy radiotherapy.[20, 22]

BODY

As noted in our original Statement of Work, we planned to complete Task 1 and begin Task 2 in Year 1 of the grant. Since we encountered some difficulties growing the multicellular layers (MCLs) from the MDA-MB 231 breast cancer cells, we had to alter the methods used in Task 1 and Task 2. Nevertheless, we were able to make progress on both of these aims, although they have not yet been completed. We also began work on the in vivo model that will be used in Tasks 3 and 4. Details of our progress on each specific task are given below.

Task 1. To determine the transport parameters and cellular uptake of MKT-077 in a model of human breast cancer parenchyma at different drug concentrations and oxygen levels (Months 1-7):

Model: We will use an in vitro 3-D model of breast tumor parenchyma, the multicellular layer (MCL), grown from human MDA-MB 231 breast carcinoma cells.

Methods: MCLs will be exposed to media bubbled with different amounts of oxygen. We will add different concentrations of MKT-077 to one side of the MCL and take samples of the media from the other side at different times. The level of MKT-077 in the samples will be determined spectrophotometrically, and the time-concentration data will be fitted to a mathematical model to determine important transport parameters, e.g., the MKT-077 diffusion coefficient and the MKT-077 cellular uptake rate.

Objective:

1) Determine the effect of oxygen level on MKT-077 transport parameters and cellular uptake rate of MKT-077 as a function of drug dose in breast cancer MCLs.

Progress on Task 1: As noted above, we originally planned to determine transport parameters and uptake of MKT-077 by MDA-MB 231 cells by using the MCL model of tumor parenchyma. We spent most of the first 5 months of the grant trying to perform these transport experiments. In these

experiments, we grew 3-D slabs of MDA-MB 231 cells (multicellular layers or MCLs) on collagen-coated culture plate inserts. The insert was then placed in a specially constructed holder that allowed the lower side of the insert membrane to contact heated media underneath the insert. Then we placed a known concentration of MKT-077 in the well of the insert (chamber 1), and measured the concentration increase in media on the other side of the insert membrane (chamber 2). The plan was to fit these data to a diffusion model to calculate the diffusion coefficient of MKT-077 in the MCLs.

We first validated the principle of the technique by measuring the transport of MKT-077 across the collagen-coated membrane of the culture plate inserts, i.e., inserts without MDA-MB 231 cells growing on them. We measured MKT-077 in chamber 2 following addition of a known concentration of the drug to chamber 1 (50-390 μg/ml). The data were fitted to a diffusion model by solving a set of partial differential equations using software written in MatLab (MathWorks, Inc., Natick, MA). An example of one such experiment and the resulting model fit are shown in Figure 1. We performed six such experiments, and the diffusion coefficient of MKT-077 across the membrane was found to be $2.21 \pm 0.38 \times 10^{-7}$ cm^2/sec (mean ± SD, n=6).

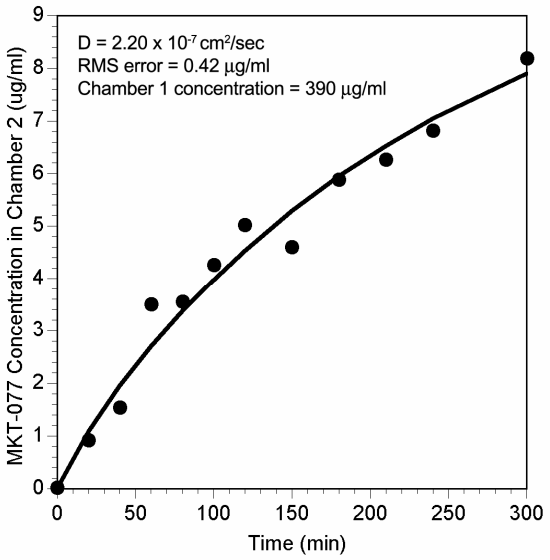

Figure 1. MKT-077 concentration in Chamber 2 following addition of 390 mg/ml MKT-077 to Chamber 1 at time zero. An insert with no MCL growing on the membrane was placed in the chamber. The curve is a least-squares fit of a diffusion model.

We simultaneously began trying to grow MDA-MB 231 MCLs on the insert membranes. Unfortunately, the growth of MCLs was very inconsistent and patchy. This was surprising, since we have been able to consistently grow uniform MCLs using three different human choroidal melanoma cell lines (OCM-1, C918, and M619). We were only able to generate data from six MCLs, but it was difficult to verify that the MCLs maintained uniform thickness throughout the experiment. An example of data from one of these experiments is shown in the lower trace in Figure 2. MKT-077 accumulation in chamber 2 is shown as a function of time after addition of the drug to chamber 1. For comparison, the two upper curves are experiments in which only the membrane was present (see above). This clearly shows that transport from chamber 1 to chamber 2 was slowed by diffusion through the MCL and possibly uptake of the drug by the MDA-MB-231 cells. The data have not been fitted to a diffusion model, because we need to first determine an uptake rate for the cells. Unfortunately, this was one of few successful experiments due to poor MCL growth with this cell line. We tried several changes in our technique to grow more uniform MCLs, but none seemed to result in better growth. I also communicated with Dr. Kevin Hicks in New Zealand, who originated this technique.[13, 14] He told me that MCL growth was tumor cell line-dependent and that he had been unable to grow MCLs adequately in several cell lines. By this time, we had attempted to grow 71 MDA-MB 231

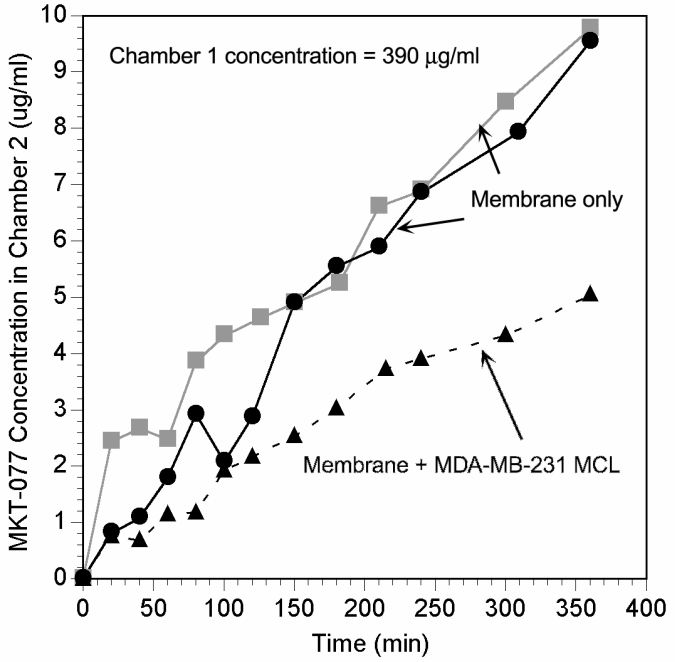

Figure 2. MKT-077 concentration in Chamber 2 following addition of 390 mg/ml MKT-077 to Chamber 1 at time zero. Either an insert with no MCL growing on the membrane (top two curves) or a membrane with a MDA-MB 231 MCL (lower curve) was placed in the chamber.

MCLs, and had generated only 6-10 MCLs that were useful or marginally useful. Given these results, we decided to complete Task 1 using a different technique.

In our opinion, one of the most important cellular parameters for this project is the uptake rate of MKT-077 by MDA-MB 231 cells. In order to know that this drug can be effective in vivo, we need to verify two key hypotheses: 1) The MKT-077 is taken up by the MDA-MB 231 cells in significant amounts and 2) the uptake rate is sufficient to decrease the oxygen consumption rate of the cells. Since we were having difficulty growing the MCLs, we decided to use a different method to determine MKT-077 uptake rate. This new technique involves growing the MDA-MB 231 cells in 6-well plates. After the cells are confluent, a known concentration of MKT-077 is added to each well. At specific times, the supernatant is removed, and the cells are trypsinized and counted. Immediately thereafter, ethanol is added to the cells to lyse them and free the MKT-077. The concentration of MKT-077 in the ethanol is measured using a spectrophotometer, and the amount of MKT-077 taken up by the cells is expressed as ng MKT-077/100,000 cells. An example of the results from one experiment, in which cells were exposed to 7.9 µg/ml MKT-077 at time zero, is shown in Figure 3a. It is clear that the cells take up the drug relatively quickly, and that the uptake rate is linear out to three hours. The fast uptake rate is important, since the concentration of drug exposed to the tumor will diminish after infusion into the rat's circulation.[21] In this particular experiment, the uptake rate is 1.08 ng MKT-077/(100,000 cells min). So far, we have performed 7 uptake experiments at several different MKT-077 doses. The results are summarized in Figure 3b. Currently, there appears to be no clear correlation between MKT-077 dose and uptake rate for the range we have studied. These experiments are ongoing and should be completed in the next few months. In the upcoming year, we plan to repeat these measurements with cells grown under hypoxic conditions and/or exposed to hypoxia during the uptake experiment. By doing this, we should be able to determine how the oxygen availability affects the uptake, which is mitochondria-dependent.

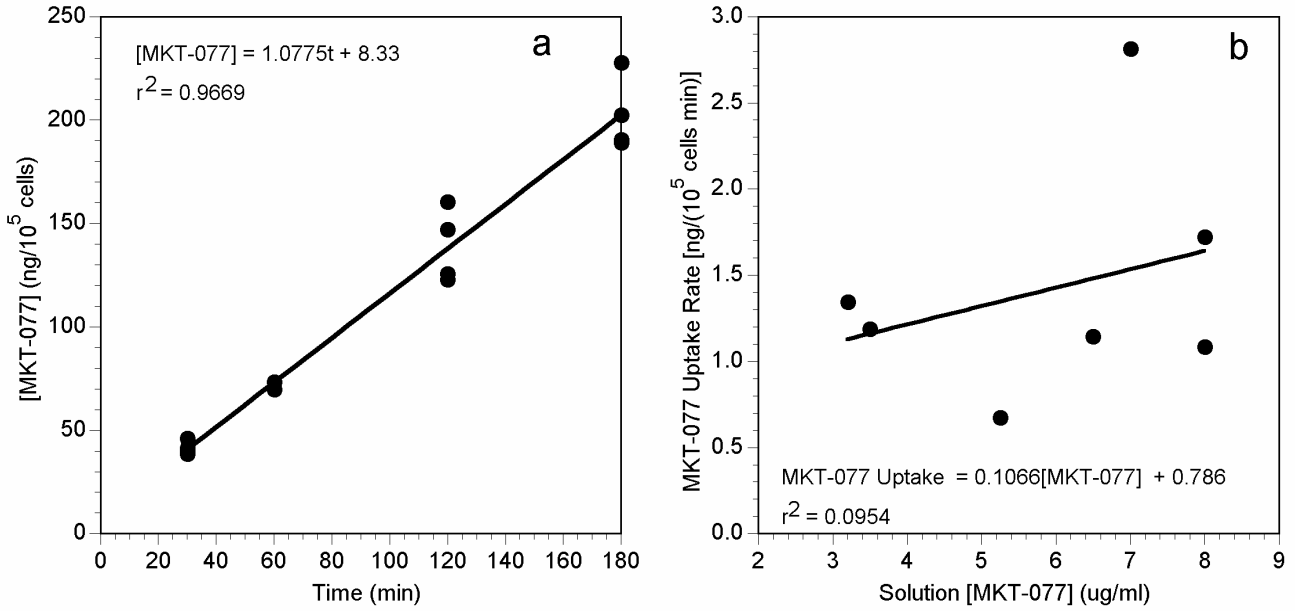

Figure 3. a. Accumulation of MKT-077 in MDA-MB-231 breast cancer cells as a function of time after addition of the drug. The MKT-077 concentration was 7.9 mg/ml. b. MKT-077 uptake rate by MDA-MB-231 cells as a function of MKT-077 concentration.

Task 2. To determine the effect of MKT-077 on tumor metabolism in a model of human breast cancer parenchyma at different drug concentrations and oxygen levels (Months 8-18):

Model: We will use an in vitro 3-D model of breast tumor parenchyma, the multicellular layer (MCL), grown from human MDA-MB 231 breast carcinoma cells.

Methods: We will use oxygen microelectrodes to measure oxygen tension (PO_2) across an MCL exposed to different concentrations of MKT-077. PO_2 profiles will be measured while the MCL is exposed to different levels of oxygen in the media. The PO_2 data will be fitted to a mathematical diffusion model to determine the oxygen consumption rate of the MCL.

Objectives:

1) Determine if exposure of MCLs to different doses of MKT-077 causes a decrease in oxygen consumption and a concomitant increase in intratumoral oxygen tension (PO_2). We predict that higher doses of MKT-077 will cause a greater decrease in oxygen consumption.
2) Determine if the MKT-077-induced decrease in oxygen consumption is a function of local oxygen level.

Progress on Task 2: Again, we originally planned to determine the effect of MKT-077 on the metabolism of MDA-MB 231 cells by using the MCL model of tumor parenchyma. As noted in the "Progress on Task 1" section, we have abandoned the MDA-MB 231 MCL model. When that was done, we decided to measure drug-induced oxygen consumption changes in the MDA-MB 231 cells using a different technique. In keeping with the original time schedule, these experiments were begun several months ago. After optimizing the system, we have now begun to gather the necessary data.

The current technique involves growing the MDA-MB 231 cells to confluency in tissue culture. The cells are then trypsinized, counted, and resuspended in fresh tissue culture media to a concentration of 1 x 10^6 cells/ml. Four ml of the suspension are placed in the inner chamber of a specialized tonometer that is heated to 37^{o}C by circulating warm water. A Clark-type oxygen electrode (InO2 Dissolved Oxygen System, Innovative Instruments, Inc., Tampa, FL) is positioned in the suspension within the inner chamber. The suspension is exposed to air and allowed to equilibrate by gently stirring the suspension with a small magnetic stir bar. The chamber is then sealed, and oxygen tension (PO_2) is recorded continuously. After 20 minutes, concentrated MKT-077 is added to the inner chamber to bring the drug concentration to the desired level. The recording is continued for at least another 40 minutes. An example of one such experiment is shown in Figure 4. In this experiment, the rate of decline in PO_2 changes within 20 minutes after addition of MKT-077 (4.2 µg/ml). By fitting the three segments of the data to straight lines using linear regression, we can determine the slopes (dP/dt) before addition of the MKT-077, immediately after addition of the drug, and 20 minutes after addition of the drug. In this case the three slopes were -0.4677, -0.4684, and -0.3487 mm Hg/min, respectively. Assuming an oxygen solubility of 3.0 x 10^{-5} ml O_2/(ml suspension mm Hg) and accounting for the concentration of cells, the corresponding oxygen consumption rates were 1.40 x 10^{-6}, 1.41 x 10^{-6}, and 1.05 x 10^{-6} ml O_2/(10^5 cells min). The consumption rate initially remained unchanged immediately after addition of the MKT-077, but it dropped by 25% approximately 20 minutes after addition of the drug. Most likely, this time is needed for the drug to be taken up by the cells and to localize to the mitochondria.

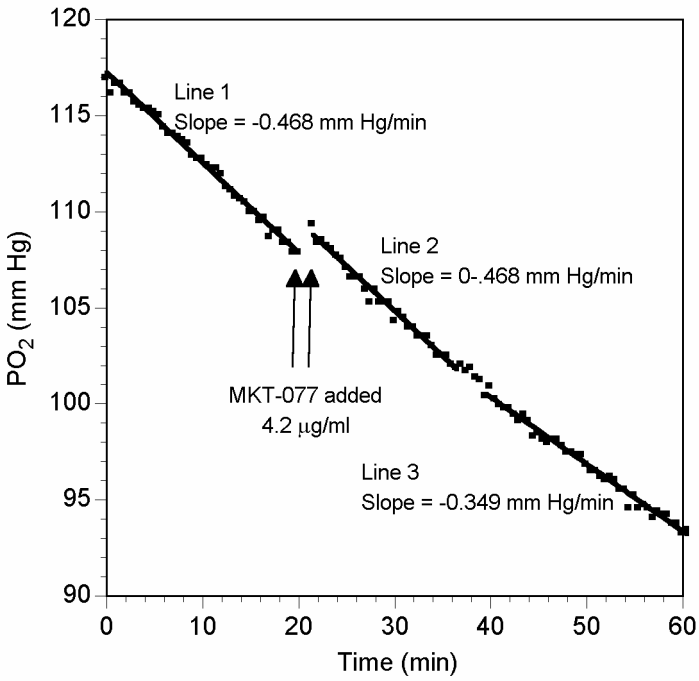

Figure 4. PO_2 measured in a MDA-MB 231 cell suspension before (Line 1) and after (Lines 2 and 3) addition of MKT-077 to the media. The final MKT-077 concentration was 4.2 µg/ml.

Since the time course of drug uptake and drug effectiveness will be important for our in vivo studies, we have also used a more complex mathematical model to examine the data. We first assume that the oxygen consumption rate, q, changes as a given function of time. As a first approximation, we have assumed that q changes monoexponentially from q_1 to q_2. The resultant function can be substituted into the mass balance equation for this system: $k(dP/dt) = -Cq(t)$, where k is the oxygen solubility of the suspension (ml O_2/ml suspension/mm Hg), P is the PO_2 (mm Hg), t is time (min), C is the concentration of cells (10^6 cells/ml suspension), and q(t) is the oxygen consumption rate as a function of time. By integrating this function, PO_2 can be expressed as a function of time and four unknown parameters, including q_1, q_2, and a time constant term (tc). Using MatLab software (MathWorks, Inc., Natick, MA), we have written a program to fit the experimental PO_2 data to this model. The results of the fit to the example data from Figure 4 are shown in Figure 5a, while the postulated changes in q are shown in Figure 5b. Clearly this assumption fits the data well, and the model shows that q changes rapidly following addition of the MKT-077. The values of q_1 and q_2 are 1.63×10^{-6} and 0.89×10^{-6} ml $O_2/(10^5$ cells min), respectively. The consumption at 40 minutes is 1.00×10^{-6}, which has not yet reached the proposed final consumption value of 0.89×10^{-6} ml $O_2/(10^5$ cells min). The time constant (tc) from the monoexponential fit is 19.6 minutes. We will continue to fit subsequent data to this model in order to determine the time course of the consumption changes. In addition, we plan to test different assumptions for the temporal changes in the consumption term, including a sigmoidal time course.

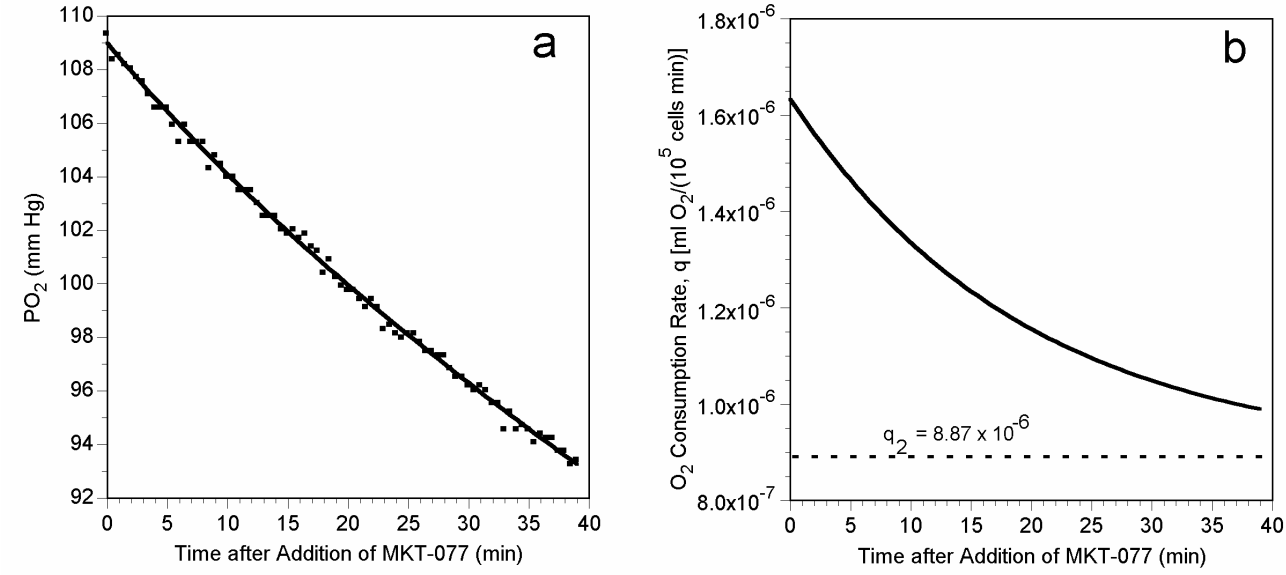

Figure 5. a. PO_2 measured in a MDA-MB 231 cell suspension after addition of MKT-077 to the media at time zero. The final MKT-077 concentration was 4.2µg/ml. The solid line is a least-squares fit of the data, assuming that oxygen consumption decreases monoexponentially. b. The monoexponential drop in consumption, q, as determined by the fit: $q = q_2 - (q_2 - q_1)e^{-t/tc}$. The parameter values are given in the text.

After optimizing the system, we have performed three successful experiments so far. Exposure of the cells to 4.2 µg/ml of MKT-077 decreased oxygen consumption from $1.79 \pm 0.43 \times 10^{-6}$ to $1.39 \pm 0.55 \times 10^{-6}$ ml $O_2/(10^5$ cells min) (mean $\pm$ SD, n=3). This is an average decrease of 22% (paired t-test, p=0.077, n=3). Two more experiments will be performed at this dose, and then we will determine the consumption change caused by other doses of MKT-077. We are slightly behind the proposed schedule for these experiments, but we should complete them by Month 18, as originally projected. This should be possible, because these experiments are technically less challenging than the MCL experiments that we originally proposed. As was the case for the experiments in Task 1, we would like to expand these to test the effects of hypoxia on the magnitude and time course of the MKT-077-induced change in oxygen consumption.

Task 3. To determine if MKT-077 infusion can increase tumor PO_2 in orthotopic xenografts grown from human breast cancer cells without altering tumor blood flow (Months 19-33):

Model: We will grow MDA-MB 231 human breast carcinomas orthotopically as xenografts in the mammary fat pads of female nude, athymic rats. We estimate requiring 120 rats for this study.

Methods: We will use oxygen microelectrodes to measure PO_2 at a single site in the xenograft following infusion of different doses of MKT-077. Tumor blood flow will be measured simultaneously at multiple sites using laser Doppler flowmetry to see if MKT-077 affects tumor perfusion. In a second set of experiments, we will measure PO_2 histograms in the xenografts either 2 or 24 hours after infusion of MKT-077 and determine the median PO_2 and other characteristics of the overall distribution.

Objectives:

1) Determine if MKT-077 infusion will transiently increase PO_2 in orthotopic breast tumors without altering local blood flow in a dose-dependent manner (Months 19-23). These are important measurements, since changes in perfusion can greatly affect tumor PO_2.
2) Determine if MKT-077 infusion will increase the median PO_2 in orthotopic breast tumors in a dose- and time-dependent manner (Months 24-33).

Progress on Task 3: As noted above, these experiments are not scheduled to begin until the middle of the upcoming funding period (Year 2). Nevertheless, because of the initial difficulty with the in vitro MCL work, we began to work with the nude rat model a few months ago. Unexpectedly, we have had difficulty getting MDA-MB 231 xenografts to grow in the mammary gland of female nude rats. We have attempted to grow tumors in five Hsd:RH-rnu/rnu nude rats. In three rats, we injected 0.1 to 0.15 ml of MDA-MB 231 cell suspensions, containing a total of 1×10^6, 2×10^6, or 6×10^6 cells. None of these injections resulted in tumor growth, even though they were followed for at least five weeks. In the other two rats, we injected large pellets of tumor spheroids that we had grown in culture. We did not have accurate cell counts on these, but we estimate that they contained at least 5×10^6 cells. Since we have had success growing human choroidal melanomas orthotopically in the eyes of nude rats using spheroids,[4] we thought injection of spheroids might result in MDA-MB 231 tumor growth in the mammary gland. Unfortunately, no tumor growth was evident following implantation of MDA-MB 231 spheroids into the mammary glands of the nude rats. Others have had success growing MDA-MB 231 xenografts in nude mice by injecting cell suspensions,[2, 9, 10] so we did not anticipate this difficulty.

In the near future, we will attempt to implant MDA-MB 231 cells enmeshed in Matrigel®, which was found to enhance growth of MDA-MB 231 tumors in nude mice.[1, 17] If we cannot get these cells to form xenografts, we have access to another human line, MDA-MB 468, that has been successfully grown in nude animals.[3, 15] If all of that fails, our final fallback model will be the orthotopic R3230Ac rat mammary adenocarcinoma growing in the mammary gland of the Fischer 344 rat. We have had experience growing this tumor in Fischer 344 rats before.[5, 6] Ideally, we would like to perform these experiments with the MDA-MB 231 cells. Therefore, we will make every effort to get these xenografts to grow. Nevertheless, the proposed experiments will be performed in an orthotopic breast cancer model, even if the MDA-MB 231 cells do not form tumors. In the meantime, we have been performing studies in the nude rats to better determine the MKT-077 dosages and infusion rates that we can use in future experiments. In these experiments, Hsd:RH-rnu/rnu rats were anesthetized with pentobarbital (50 mg/kg IP), and the femoral artery and vein were cannulated for monitoring of blood pressure and drug infusion, respectively. In the first rat, a dose of 15 mg/kg MKT-077 was infused intravenously as a long bolus (over approximately 1.5 minutes). The blood pressure dropped precipitously from 97 mm Hg to 48 mm Hg after 2.5 minutes. It slowly recovered to 79 mm Hg by 35 minutes after the infusion, but remained at that level for at least another 20 minutes. Based on this initial experiment, we decided to infuse the drug more slowly. In a series of three experiments, we

infused three different doses of MKT-077 intravenously at a rate of 1.25 mg/kg/min (0.5 ml/kg/min of a 2.5 mg/ml MKT-077 solution). When 7.5 mg/kg MKT-077 was infused over 7 minutes, there was an initial transient drop in blood pressure (Figure 6, thick line). Then the pressure recovered and exceeded the preinfusion value. Some of this behavior may have been a response to the drug infusion, since we noted that the anesthesia level was not as deep as we desired (there was a blood pressure response to a toe pinch). When 10 mg/kg MKT-077 was infused over 8 minutes, there was no change in mean arterial blood pressure (Figure 6, thin black line). Following infusion of 15 mg/kg MKT-077 over 12 minutes, the pressure dropped from around 110 mm Hg to a nadir of 55 mm Hg several minutes after the end of the infusion (Figure 6, dashed line). The pressure recovered to 70-80 mm Hg, but it remained decreased for the remainder of the experiment. These experiments show that we can safely infuse up to 10 mg/kg of MKT-077 into this nude rat strain without compromising systemic blood pressure. We plan to try a 12 mg/kg dose in the near future. In our upcoming experiments, we will most likely use 7.5 mg/kg MKT-077 as the low dose and 10-12 mg/kg MKT-077 as the high dose in the experiments proposed in Task 3.

Thus, we are making progress on this aim, and hope to begin these experiments early in the second year of the grant, as originally proposed.

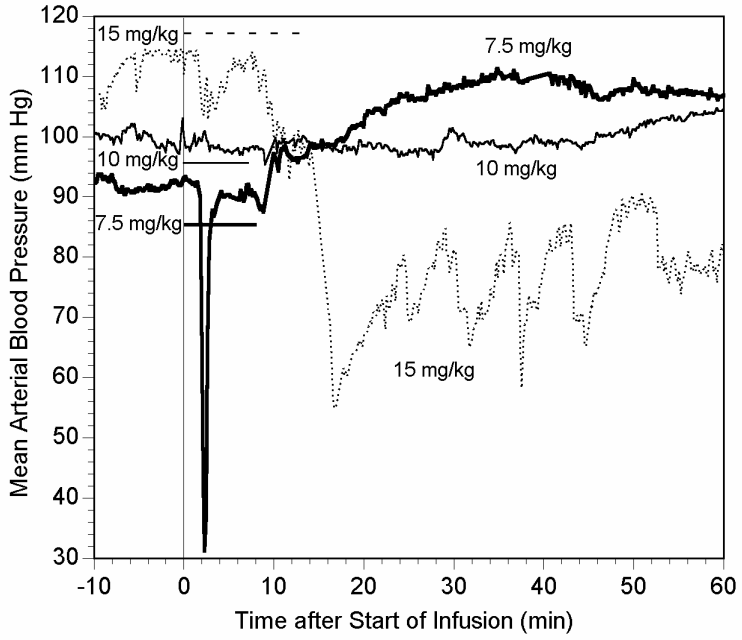

Figure 6. The effect of IV MKT-077 infusion on mean arterial blood pressure in nude rats. Either 7.5 (thick line), 10 (thin line), or 15 (dashed line) mg/kg MKT-077 was infused at a rate of 1.25 mg/(kg min). A 2.5 mg/ml solution of drug in saline was infused at a rate of 0.5 ml/(kg min) for 7, 8, or 12 minutes, respectively (see horizontal lines extending from zero line).

Task 4. To determine if MKT-077 infusion before single-dose radiation therapy can delay the growth of orthotopic human breast cancer xenografts better than either radiation or drug treatment alone (Months 34-36).

Model: We will grow MDA-MB 231 human breast carcinomas orthotopically as xenografts in the mammary fat pads of nude, athymic rats. We estimate requiring 45 rats for this study.

Methods: We will irradiate established MDA-MB 231 tumors that have either received MKT-077 or the saline vehicle. Parallel groups will be sham irradiated. Tumor volume will be measured 3 times per week until the tumors have reached 5 times the initial volume or for a maximum of 60 days.

Objective:

1) Determine if MKT-077 infusion before irradiation will result in significant tumor growth delay compared to the other three groups: saline with sham irradiation, MKT 077 with sham irradiation, and saline with irradiation.

Progress on Task 4: In the original Statement of Work, these experiments are not scheduled to be performed until Year 3 of the grant. We believe this is still a reasonable goal. In the upcoming year (Year 2), we should be able to determine a dose of MKT-077 that results in an increase in tumor oxygen levels in our rat model of human breast cancer.

KEY RESEARCH ACCOMPLISHMENTS

- We have shown that MKT-077 is readily and rapidly taken up by the MDA-MB 231 breast cancer cells.
- We have also shown that doses as low as 4 μg/ml MKT-077 are capable of quickly inhibiting oxygen consumption in the MDA-MB 231 cells. This means that we may see oxygen changes in the tumor soon after MKT-077 infusion.
- We have shown that doses of MKT-077 up to 10 mg/kg can be infused intravenously at a rate of 1.25 mg/kg/min without affecting blood pressure in nude rats.

REPORTABLE OUTCOMES

Due to the difficulties with the MCL model, we have not yet published any results on the inhibition of cellular respiration by MKT-077 in vitro. We hope to complete this portion of the work in the summer and publish a manuscript based on that work.

CONCLUSION

In this first year of the grant, we have demonstrated that MKT-077 is rapidly taken up by the human breast cancer cell line, MDA-MB 231. We have also shown that the drug is capable of inhibiting oxygen consumption in vitro in this cell line. Finally, we have also demonstrated that the drug can be safely infused intravenously into the nude rat if it is infused over time.

These results are important for several reasons. First, it validates that MKT-077 can be taken up by MDA-MB 231 human breast cancer cells. Previous studies showed that carcinoma cells preferentially take up MKT-077 compared to other cell types,[7, 8] but it was not certain that the MDA-MB 231 cells would take up the drug. Second, it is significant that the cells took up the MKT-077 rapidly and that the drug almost immediately decreased the oxygen consumption. In Task 3 we plan to measure tumor oxygenation shortly after and long after infusion of the drug into the rat. The current results suggest that MKT-077 may be effective at raising oxygen levels in the short term. It will be important to determine when the oxygen change is maximal following drug infusion. This will guide the radiation study in Task 4 and would suggest a protocol for testing in humans should MKT-077 prove to be a successful radiosensitizer in the rat. Third, 4 μg/ml of MKT-077 was able to decrease oxygen consumption in MDA-MB 231 cells by about 25%. Secomb and coworkers demonstrated theoretically that inhibition of consumption by 30% was sufficient to eradicate hypoxia in a model tumor.[18, 19] Thus, MKT-077 is able to inhibit MDA-MB 231 metabolism to an extent that is physiologically relevant. Finally, the preliminary dosing studies that we have performed have allowed us to determine the proper infusion protocols for our future studies in rats.

Although we had some difficulties getting the MCLs to grow, the subsequent in vitro methods have shown that we can determine the parameters necessary to complete this work. In the second year, we will continue to use the modified techniques to measure MKT-077 uptake under physiologically relevant conditions, and we will continue to study the kinetics of MKT-077-induced changes in oxygen consumption. In addition, we plan to begin the in vivo work to determine if MKT-077 can actually be used to raise oxygen levels in orthotopic tumor xenografts.

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APPENDICES

None.